# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



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| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT TYPE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 31/08/2013 | Final | June 1, 2010 - May 31, 2013 |

**4. TITLE AND SUBTITLE**
Electrogelation of Biopolymers for New Functional Materials

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
FA9550-10-1-0172

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
OFC OF Naval Rsch (ONRRO) Boston
Boston Regional Office
495 Summer Street, Rm 627
Boston, MA 02210-2109

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION/AVAILABILITY STATEMENT**
A = Approved for public release; distribution is unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
In this award we build on our platform of fundamental insight and technological impact related to biopolymer-based materials, to generate a new family of functional biopolymer material systems. The focus was on new observations surrounding the process of electrogelation (e-gel formation), as a novel mechanism to control polymer assembly – surface adhesion, hydrogelation and related features. We elucidated the mechanistic basis for this process and pursued further control of the material assemblies and functional features by studying the impact of a range of environmental factors. We will also extended

**15. SUBJECT TERMS**
silk, materials, electrogelation, egel, biopolymers, tropoelastin

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | David L. Kaplan |
| U | U | U | SAR | 3 | 19b. TELEPHONE NUMBER *(Include area code)* 617-627-3251 |

**AFOSR: Final Report - Electrogelation of Biopolymers for New Functional Materials [FA9550-10-1-0172]**

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**Abstract** (from the original proposal) - In this proposal we build on our platform of fundamental insight and technological impact related to biopolymer-based materials, to generate a new family of functional biopolymer material systems. The focus for the renewal will be on the new observations surrounding the process of electrogelation (e-gel formation), as a novel mechanism to control polymer assembly – surface adhesion, hydrogelation and related features. Based on preliminary studies a novel way to regulate the assembly, disassembly (reversibility) and functions of silk protein polymers was identified. We plan to elucidate the mechanistic basis for this process and pursue further control of the material assemblies and functional features by studying the impact of a range of environmental factors. We will also extend the process to other biopolymer systems to determine applicability and utility. For example, we will build a library of related block copolymer elastin-silk systems to evaluate e-gel impact on assembly and material functions. Further, we will explore the application space for these new materials, with a focus on surface coatings, adhesion with concepts in areas of dynamic surfaces or biomimetic self-repair surfaces and biosensor functions. We will also incorporate other dynamic biopolymer material systems to continue to expand fundamental insight into new systems and link mechanism to potential areas of utility for DoD and broader needs.

**Aim #1 – E-gel System for New Functional Materials**
**Aim #2 – Dynamic System to Evaluate e-gel Properties**
**Aim #3 – E-gel Mechanisms**

## Progress

### Aim #1 – E-gel System for New Functional Materials

We concluded our efforts to examine new materials from egel systems. These later studies focused on additional gel formation under different environmental conditions (e.g., salts, concentration, temperature), different morphological control to generate not just gels but also films, and different ways to functionalize the films (such as the inclusion of beads and particles). In all cases the gels or films were studied for formation and reversibility in response to polarity and reaction conditions. These results built off of the three year effort to exploit egel systems for new materials and these studies have been published or are currently being completed for publication. The significance of this effort is in understanding where and how to best utilize egels based on the conditions for formation, reversibility and function.

The further studies with a focus on morphology of the e-gel features were submitted for publication. We completed these morphological studies that we first reported on a few months ago. Besides the gelation mechanism of ion migration influencing the spatial distribution of pH, the α-helices in silk serve as electric dipoles and align along the direction of the electric field. We tuned covalent and physical β-sheet crosslinks in silk hydrogels in order to preserve the morphology of the material after critical point drying. Scanning electron microscopy (SEM) of glutaraldehyde-crosslinked, ethanol dehydrated, silk e-gels revealed locally aligned fibrillar structures. Fourier transform infrared spectroscopic analysis of electrogelled, vortex-induced and spontaneously formed silk hydrogels showed that the secondary structure of silk *e*-gels was tunable between non β-sheet dominated and β-sheet dominated states. Dynamic oscillatory rheology was used to investigate the mechanical reinforcement of silk *e*-gels provided by controlled chemical and physical crosslinks.

We have also continued to design additional biopolymers with utility to exploit the egel process. We have focused on the silk and tropoelastin systems due to our ability to genetically engineer such system with designer features. We have recently shown that combinations of silk and tropoelastin will permit egel formation on both poles. We see some of these new protein designs as possible new adhesive systems with responsive properties, by combining the features of silks and tropoelastin in new ways. We have examined the mechanical, thermal and responsive properties of these new combinations of proteins and the studies are currently being written up for publication. Since protein polymers are tunable we have developed a platform approach for rapid synthesis, high-throughput screening and functional testing of diverse stimuli-responsive protein materials as we have mentioned in our last report. The utility of the approach was demonstrated by synthesizing and screening variants of silk-elastin-like polypeptides. We have been screening the library for a range of environmental triggers, from the more traditional inverse temperature-based approach, to pH and oxidation-reduction approaches, while also looking for electrogelation responses. The proteins are being analyzed in various forms and assess for transitions for responsiveness.

We have also focused efforts on exploiting silk matrices for stabilization of bioactive components as we reported in the program last year. In our most recent efforts, we have expanded the set of proteins that have been stabilized in the silk matrices, as well as our ability to sequester genetic materials. These features expand the potential utility of these systems above and beyond just simple drugs, into the realm of sample collection and storage for a variety of archival and related needs, as well as for biosensor related functions. These findings have significant implications in the general use of silk materials for the entrainment, stabilization and use in a range of applications. For example, in collaboration with the Air Force Materials Lab as reported last year, organophosphates, were studied. Organophosphates are some of the most acutely toxic compounds synthesized on an industrial scale, and environmental contamination by these compounds is of great concern for human health as well as aquatic ecology. Organophosphorus hydrolase (OPH) activity is vulnerable to environmental conditions that would accompany its practical utility; a limitation that can also be extended to conditions required for incorporation of OPH into useful materials. We found that entrapment of OPH in silk films leads to stabilization of OPH activity under a variety of conditions that otherwise reduce activity of the free enzyme. Resistance to long-term storage was increased when OPH was entrapped in silk fibroin, particularly when stored dry. Additionally, silk fibroin entrapped OPH demonstrated increased stability under adverse environmental conditions such as elevated temperature, UV light exposure and exposure to high detergent as well as organic solvents. The chemical resistance observed with silk fibroin-entrapped OPH allowed for its dispersal into a polyurethane-based coating that retained organophosphate hydrolysis activity after formulation, application and drying. The data demonstrate the utility of silk fibroin-OPH entrapment for the enhancement of activity as well as a basis for the construction of robust, multifunctional materials designed for protection, detection and decontamination.

**<u>Aim #2</u> – Dynamic System to Evaluate e-gel Properties**

As reported previously, we have designed and implemented microfluidic flow chambers with embedded electrodes in order to study the dynamics of egel formation. These designs have enabled us to assess formation of egel under flow, reversibility and to embed particles to assess formation. In our most recent effort we assessed fluid shear experiments to examine interfacial behavior, gel deformation and recovery in the devices. These data are being combined with the prior experiments and being written up for publication. The significance of this effort is as a new opportunity to use egel formation and reversibility as a mode for material coatings that would be reversible, such as for living skins or for drag reduction.

**<u>Aim #3</u> – E-gel Mechanisms**

We have continued to study mechanisms involved in egel formation and adhesion. These more recent studies focused on salt shielding, hydrophobic interactions and material surfaces factors. We have a clearer picture from the work on the project of the role of pH, but also insight into the role of other factors such as the morphology and structure of the gels, the role of various additives on gelation and the adhesion. These studies built off of our modeling efforts which we reported last year for the project and are currently being completed for publication. The electrodiffusion model for silk gelation under electric fields was published and involved local pH changes as a result of water electrolysis – generating $H^+$ and $OH^-$ ions at the (+) and (-) electrodes, respectively, as we reported this past year. Silk fibroin has a pI=4.2 and when local pH<pI, e-gel forms. A finite-element ion electrodiffusion model was developed and tested and relied on electrodiffusion of the generated $H^+$ and $OH^-$ ions. Initially, inputs into the model were the measured e-gel and voltage curves. The governing ion electrodiffusion equations were solved and the calculated pH matched the experimental pH profile, indicating that ion electrodiffusion dictates local pH changes and E-gel growth. Furthermore, the model predicted the constant currents (2 mA and 3 mA) necessary for two hypothetical e-gel growth curves and these results were then validated experimentally. The model thus shows how ion electrodiffusion governs the electrogelation process and also provides predictable outcomes for fundamental and practical E-gel applications.

**Interactions/Transitions**

Rajesh Naik - Air Force Materials Lab – OPH stabilization in silk, structural characterization of silk materials
Vladimir Tsukruk – Georgia Tech – new materials from silk materials including ionomers and gels

**Funding Profile:**

| FY11 | FY12 | FY13 |
|---|---|---|
| $155,740 | $159,842 | $282,106 |

**Publications:**


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